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Award Number: DAMD17-96-1-6202

TITLE: Lifetime Alcohol Exposure and Breast Cancer Risk

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REPORT DATE: October 2001

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2001	Final (15 Sep 96 - 14 Sep 01)

4. TITLE AND SUBTITLE
Lifetime Alcohol Exposure and Breast Cancer Risk

5. FUNDING NUMBERS
DAMD17-96-1-6202

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

This case-control study was an examination of breast cancer risk in relation to lifetime alcohol consumption. We interviewed 1,181 women with breast cancer (295 pre- and 886 post-menopausal women), age 35-79, from Erie and Niagara counties in New York State, all with incident, pathologically confirmed breast cancer. A total of 2,181 controls were interviewed; controls were randomly selected and frequency matched to cases on age, race and county of residence. Participants complete a computerized interview, which focused on in-depth lifetime alcohol consumption history. Potential confounding factors were also assessed. A specimen bank is being used to store biological samples for future research of serum and urinary markers of hormones, hormone metabolites, vitamins, genetic polymorphisms and blood levels of antioxidants and oxidative stress. At the completion of the study, 969 blood samples were stored for cases and 2,016 for controls. Data collection has been completed. Some data analysis has been completed as well and preliminary findings are included. Data analysis will continue to utilize this excellent resource.

14. SUBJECT TERMS
Breast Cancer, alcohol consumption, alcohol dehydrogenese, epidemiology

15. NUMBER OF PAGES
17

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

This research was an epidemiologic investigation into the role of lifetime alcohol exposure in breast cancer etiology, research of considerable relevance to the issue of breast cancer prevention, providing insight on the role of a modifiable, and common exposure. The primary purpose of this study was to examine the history of alcohol consumption from adolescence through adulthood as a risk factor for pre- and post-menopausal breast cancer in women. We also plan to examine the possible role of factors such as genetic factors, estrogen receptor status, histology, and use of estrogen replacement therapy among post-menopausal women in mediating the effect of alcohol on breast cancer risk. In this case-control study, we interviewed 1,181 women (295 premenopausal and 886 postmenopausal), age 35-79 from Erie and Niagara counties in western New York with incident, pathologically confirmed cases of breast cancer. We also interviewed 2,181 controls (600 premenopausal and 1,581 postmenopausal). Controls were randomly selected, those under age 65 from lists provided by the New York State Department of Motor Vehicles, those age 65 and over from enrollment lists of the Health Care Finance Administration. Controls were frequency matched to cases on age, race, and county. Interviewing of cases and controls was completed in June 2001. Blood samples are being stored in a biological specimen bank for future research. We present here preliminary findings from this study regarding lifetime alcohol intake and risk of breast cancer. Previous research in this area has relied on relatively crude measures of alcohol consumption, generally not distinguishing infrequent drinkers of larger amounts from individuals who drink smaller quantities more often, and those who drink with meals from those who do not drink with meals. There is some evidence that age when drinking began may also affect risk; studies have not examined in detail characteristics of early drinking. Using the data collected from this study, we will be able to examine drinking pattern in relation to risk. Further, there is some evidence that women may differ in their metabolic response to alcohol depending on genetic polymorphisms for enzymes involved in alcohol metabolism. We published a report based on an earlier case-control study of breast cancer that indicated that there were differences in risk by the genotype of alcohol dehydrogenase 3 for pre- but not postmenopausal women (1). We now have preliminary evidence from this study that confirms that finding. With the interviewing now completed, we will be able to look into that finding in more detail because the new study includes a very detailed lifetime alcohol history. We will soon begin to analyze and make presentations on the data. We also have had two new grants funded utilizing and enhancing this data set. We have submitted another as well that is pending.

(5) Body of Report

Task 1: Months 1-3: Obtain Institutional Review Board approval for the study at all area hospitals

This task is completed. Annual progress reports are submitted to each hospital and IRB approval has continued unchanged. We obtained approval from the Institutional Review Boards of twelve hospitals in the region. One exception was Sisters' Hospital, a major hospital, which would not allow us to use their patient population in spite of long negotiations and considerable effort on the part of both our group and the hospital Institutional Review Board. However, the practice of breast surgeons who see virtually all of the breast cancer patients at that hospital cooperated fully with the study and allowed us to contact patients using their clinic records.

Task 2: Months 1-3: Finalization of all arrangements for interviewing: training interviewers, necessary preparations of computer interview, printing of the paper section of the questionnaire, obtaining lists of potential controls from the Department of Motor Vehicles and the Health Care Finance Administration, purchase of all necessary supplies and equipment.

This task is completed. Our trained staff completed all interviews of cases and controls in June 2001.

Task 3. Months 4-45. During years 3 and 4 we will interview 700 cases, making a total of 960 (approximately 215 premenopausal and 745 postmenopausal women). We will continue interviews of postmenopausal white women in years three and four. In addition, we will interview 150 controls.

Cases:

This task is completed. We interviewed a total of 1,181 breast cancer cases (295 pre-menopausal and 886 post-menopausal). A no cost extension on our grant allowed us to surpass our projected total of 960 case interviews. The additional interviews are important for increased power of the study, particularly for the examination of gene-environment interactions.

We ascertained cases by having nurse-casefinders visit hospitals at frequent intervals, the frequency determined by the patient load in that hospital. They examined pathology department records to obtain names of individuals aged 35-79 with a histologically confirmed diagnosis of primary breast cancer, living in Erie and Niagara counties and with no previous history of cancer. When a case was found, we mailed a form for permission to interview to the physician, requesting that the physician indicate approval. In addition, this letter requested verification from the physician of the diagnosis of primary breast cancer. If there was delay, we contacted the physician by phone. Once approval was obtained, we invited the patient to participate. Most cases were interviewed within 6 months of diagnosis; none were interviewed more than a year after diagnosis. We secured informed consent from all subjects.

We have compared our case ascertainment with data from the New York State tumor registry. Because there is considerable delay in obtaining registry data, those data are not an appropriate tool for identification of cases. We have used the tumor registry to verify the completeness of our case identification. Based on statistics for 1993-7, the number of cases identified in our study are very comparable to those of the tumor registry, exceeding those statistics slightly.

Age and racial distribution of the cases interviewed for this study are provided in detail in Table 1.

Table 1. Characteristics of Breast Cancer Cases Interviewed

Age	Caucasian	African-American	Other Racial Groups
35-44	129	17	0
45-54	286	23	6
55-64	314	21	3
65-74	265	13	2
75-84	95	6	1
Total	1089	80	12

The overall participation rate for cases for the ongoing case-control study to date (as of 9/00) is approximately 40%. This participation rate includes individuals with incident breast cancer who were alive and eligible for the study, and whom we were able to contact. Numerous attempts were made to maximize participation rate including working closely with area physicians who have been, on the average, quite supportive of our efforts. Overall approximately 11% of the refusals are from the physician. We have worked extensively with local physicians to obtain good participation. We have worked closely with some of the breast surgeons and oncologists who see most of the breast cancer patients in order to attain good physician and patient participation.

It should be noted that we believe that our recruitment rate compares very favorably with other case-control studies conducted in the general population that require an extensive visit and blood drawing. Higher participation, at least in the U.S., has been reached only in studies where participants were interviewed by telephone, in hospitals or with minimal interview time or no blood drawing. The Department of Social and Preventive Medicine and the investigators involved in the proposed studies have long-term and successful experience in recruiting for epidemiological and clinical trial studies. We are part of the Western New York Vanguard Clinical Center of the Women's Health Initiative (WHI). The Western New York Center was the first nationwide (among 40 clinical centers) to meet recruitment goals for both the observational and clinical trial components of the study.

Table 2. Characteristics of participating and non-participating breast cancer cases completing a brief telephone questionnaire

	Participants (n=272)	Non-Participants (n=120)
Age (mean yrs ± sd)	58.8 (11.0)	64.8 (10.5)
Race (%)		
White	94	91
African American	5	8
Other	1	1
Education(mean yrs ± sd)	13.7 (2.3)	13.1 (2.4)
Alcohol Intake (drinks/mo)	3.8 (5.5)	3.1 (5.1)
Vegetable Intake	10 (4)	9 (4)

In order to assess the bias related to non-participation, we conducted a short interview with both those participating and those not participating, at the time of telephone contact, in order to characterize the non-respondents and compare them to those who did participate. All of those contacted were asked a small number of questions. Not all of these data have been entered; data that are available are presented here. Comparisons of responses of participating and non-participating breast cancer cases are shown in Table 2. In general, participants and non-participants completing this survey were similar. Participants were somewhat younger and more educated. There was little difference between the groups in either alcohol intake or vegetable intake.

Controls:

This task is completed. We interviewed a total of 2,181 controls interviewed (600 pre-menopausal and 1,581 post-menopausal), again surpassing our original goal of 2030 controls (685 pre-menopausal and 1,345 post-menopausal).

Controls under age 65 were randomly selected from the list of those holding driver's licenses in Erie and Niagara counties; controls age 65 and over were randomly selected from the rolls of the Health Care Finance Administration. Excluded were women with a history of cancer other than non-melanoma skin cancer. Controls were frequency-matched to cases on age, race, and county of residence.

Characteristics of controls are shown in Table 3.

Table 3. Characteristics of Controls Interviewed

Age	Caucasian	African-American	Other
35-44	318	16	8
45-54	524	29	6
55-64	434	24	6
65-74	553	91	5
75-84	142	25	0
Total	1971	185	25

As for the cases, response rate is low, 37%. Again as for the cases, we have done a brief telephone interview to determine the characteristics of non-participants and participants. The available data are summarized in Table 4.

Table 4. Characteristics of participating and non-participating controls completing a brief telephone questionnaire

	Participants (n=1521)	Non-Participants (n=1123)
Age (mean yrs + sd)	58.4 (11.9)	61.4 (13.7)
Race (%)		
White	92	89
African American	7	9
Other	1	3
Education(mean yrs + sd)	13.7 (2.4)	12.8 (2.2)
Alcohol Intake (drinks/mo)	4.1 (5.4)	3.0 (4.8)
Vegetable Intake (servings/wk)	10 (4)	9 (4)

As for the cases, participants and non-participants completing this survey were very similar. Again, participating controls were somewhat younger and more educated. There was little difference between the groups in either alcohol intake or vegetable intake.

Task 4: Months 3-47: Ongoing data entry of the interview, maintenance of files from computer-assisted interview and entry of data from the sections of the interview completed by the participant.

This task is completed. All forms from the interview have been coded and entered. There was considerable coding required for several of the self-administered interviews. These include information on family history of cancer and other diseases, vitamin supplement use, diet intake and medical history. We are currently cleaning data files in preparation for analysis.

Copies of the computer interview and self-administered questionnaires were included in the Appendix of last year's report.

Task 5: Months 4-47: Maintenance of the biological specimen bank, processing of samples for immediate determinations and for storage, tracking of all samples, mapping of the freezer.

This task of processing the samples, tracking them and mapping them has been completed. However, the maintenance of the biological specimen bank of course continues, utilizing other sources of funding.

To ensure standardization of specimens collected, all blood was drawn at the same time of the day (7:00AM-9:00AM). For pre-menopausal women, blood drawings were scheduled for the luteal phase of the cycle to reduce, to the extent possible, variation in hormone levels related to the menstrual cycle. The time of the blood draw was recorded for assessment of any variation in blood markers related to the time of the draw. A total of 969 blood samples for cases and 2,016 for controls have been processed for immediate determinations and for long-term storage.

Of the cases, 82% provided a blood sample; some cases were unable or unwilling to provide a blood sample because of treatment regimens. For controls, 93% consented to provide a blood specimen. All others were asked to provide a saliva sample and there was complete compliance with this request. There are participants without any sample because at the start of the study we did not collect saliva samples. Saliva samples were obtained by the method of Lum and LeMarchand (2).

We have published several papers recently regarding the issues of obtaining and storage of biological samples (3-6). Those manuscripts were included in the annual report for last year.

Tasks 6: Months 25-48: Genetic analyses of samples: DNA extractions and determinations of genetic polymorphisms.

Blood clots for DNA extraction and subsequent genetic analysis have been removed from the freezer and shipped on dry ice to Dr. Peter Shields. We have sent a total of 2,985 samples: 969 from breast cancer cases and 2,016 from controls. These samples represent all of the interviewed breast cancer cases and controls for whom we had a biological sample. DNA extraction is ongoing in Dr. Shields' lab. He has begun the ADH3 analyses and will continue with those.

Preliminary results from a subset of the total sample have been analyzed to determine the modifying effects of ADH3 on the association between alcohol consumption and breast cancer risk; those results were included in last year's annual report.

Task 7: Months 25-48. Statistical analyses; preparation of variables from the interview and blood determination, all required analyses of the data for reports and presentations.

Preliminary statistical analyses are beginning. As indicated above, considerable effort in the past year has focused on the final stages of data entry and data coding. We are now working on managing the data, identifying outliers and data inconsistencies and implementing previously established procedures for cleaning the data. Considerable work has been done on the alcohol data, the major focus of the interview. In addition, there has been considerable work done on the interview data regarding hormone use, both oral contraceptives and postmenopausal hormones. A complete data set that has been completely readied for analysis should be available within the next two months. When final preparation of the data set is completed, we will begin the data analysis on the major aims of this grant. We plan to examine lifetime alcohol consumption and risk, examining different periods in the life cycle to determine if there are periods of greater sensitivity to alcohol consumption. We will also analyze differences in the effect of alcohol depending on drinking pattern, i.e., whether alcohol is consumed with meals or not and whether drinking is in frequent small amounts or less frequent larger quantities. We will examine the modifying effects of alcohol dehydrogenase (ADH3) genetic variants on the alcohol and breast cancer association. We will also examine modifying effects of estrogen receptor status, histology of the tumor and the use of hormone replacement therapy for postmenopausal women.

Additional analyses are also planned. We are currently funded to examine lifetime residential history as a proxy for industrial exposures in relation to breast cancer risk. We have identified, coded and entered approximately 20,000 addresses from lifetime residential histories for these women. We are currently obtaining information regarding the industrial exposures in Erie and Niagara Counties for the time period of interest. We have also obtained IRB approval to access birth certificates for the women in the study so that we can examine the effects of perinatal exposures as well as verifying their address at birth. Further, we have a grant pending to examine some additional genetic factors and tumor characteristics in relation to alcohol and other dietary factors and breast cancer risk.

Very preliminary results are shown in Tables 5-6.The descriptive information regarding the total sample is shown in Table 5. Preliminary results regarding the main effects of total lifetime alcohol and beverage specific consumption are shown in Table 6. All results are very preliminary and may change as the data set is examined more carefully. Based on these results, we do not see an increase in risk of breast cancer for total lifetime consumption of alcohol. We do see some indication of increased risk with increased beer and wine consumption.

Table 5. Descriptive characteristics of breast cancer cases and controls				
	Premenopausal		Postmenopausal e	
	Cases	Controls	Cases	Controls
Age, years	45.5* (6.2)	46.6 (8.7)	61.6* (9.5)	63.3 (9.0)
Education, years	13.9 (2.4	14.1 (2.3)	13.3* (2.6)	13.0 (2.4)
Age at menarche, years	12.6 (1.6)	12.6 (1.7)	12.6* (1.6)	12.8 (1.7)
Age at menopause, years	---	---	48.0* (5.4)	47.3 (6.3)
Age at first birth, years	20.6 (10.6)	21.4 (10.4)	19.7* (10.1)	21.1 (8.2)
Parity	1.9** (1.3)	2.1 (1.4)	2.4* (1.8)	3.0 (1.9)
History of benign breast disease	37%¶	21%	34%¶	22%
Total lifetime ounces ethanol	2046 (3167)	2657 (6426)	3099 (6020)	3372 (13400)
Lifetime ounces ethanol from beer	1637 (2643)	1692 (3810)	2257 (4240)	2272 (5857)
Lifetime ounces ethanol from wine	1070 (1593)	1317 (2784)	1818 (2872)	1634 (3946)
Lifetime ounces ethanol from liquor	1248 (2078)	1823 (5145)	2442 (5379)	2987 (13377)

All values are means (SD) except for history of benign breast disease which is percent women reporting yes; differences in means assessed with t-test, *$p<0.01$, **$p<0.05$; differences in benign breast disease assessed with χ^2, ¶$p<0.01$

Table 6. Odds ratios and 95% confidence intervals for risk of breast cancer with lifetime alcohol consumption						
	Premenopausal			Postmenopausal		
	Cases	Controls	Odds ratios (95% confidence intervals)	Cases	Controls	Odds ratios (95% confidence intervals)
Total lifetime ounces ethanol						
1 (lo thru 120)	60	160	1.00	217	360	1.00
2 (121 thru 834)	65	159	0.97 (0.63-1.49)	243	358	1.08 (0.85-1.38)
3 (835 thru 2835)	69	191	0.93 (0.61-1.41)	170	326	0.81 (0.62-1.05)
4 (2836 thru hi)	59	158	0.91 (0.59-1.41)	247	360	1.03 (0.81-1.32)
p for trend			0.16			0.51
Total lifetime ounces beer						
1 (non beer drinker)	104	358	1.00	427	860	1.00
2 (lo thru 360)	59	155	1.29 (0.88-1.90)	153	268	1.10 (0.87-1.40)
3 (361 thru hi)	61	156	1.28 (0.87-1.88)	167	266	1.19 (0.94-1.51)
p for trend			0.71			0.40
Total lifetime ounces wine						
1 (non wine drinker)	75	272	1.00	289	631	1.00
2 (lo thru 260)	73	202	1.33 (0.90-1.97)	213	375	1.13 (0.90-1.42)
3 (261 thru hi)	75	195	1.53 (1.03-2.27)	246	382	1.19 (0.95-1.49)
p for trend			0.86			0.08

Table 6, Cont'd

Total lifetime ounces liquor						
1 (non liquor drinker)	94	272	1.00	287	632	1.00
2 (lo thru 265)	68	207	0.91 (0.62-1.33)	215	371	1.18 (0.94-1.48)
3 (266 thru hi)	61	190	0.93 (0.63-1.37)	247	388	1.20 (0.96-1.50)
p for trend			0.20			0.17

Total lifetime alcohol categories defined according to quartile cutpoints in control distribution; beverage specific categories defined with referent category consisting of nondrinkers of that beverage, remaining categories split at median of control distribution; odds ratios and 95% confidence intervals calculated with unconditional logistic regression adjusting for age (years), education (years), age at menarche (years), age at menopause (postmenopausal women only), age at first pregnancy (years), parity (number of births), and history of benign breast disease (yes/no); beverage specific models further adjusted for remaining specific beverages

Task 8: Months 25-48. Preparation of publications reports and presentation of the data.

Because data collection was just recently completed, only one presentation has been made on these data. Results of the preliminary findings regarding ADH3, alcohol consumption and breast cancer risk were presented at the DoD Era of Hope meeting (Spring, 2000). The abstract and the tables from that presentation were included in the last annual report. These findings need to be considered very preliminary because they do not include the entire study sample. However, it was very interesting that we found very similar results to those of our previous study. It appears that there is an increase in risk associated with relatively low alcohol consumption in premenopausal women with the at risk genotype. When the genetic analyses are completed, we will continue these analyses with the full data set and examine the associations with different aspects of alcohol consumption (e.g., consumption at different periods of life, consumption of different beverages, patterns of drinking). We plan to present new findings from our study at the American Association for Cancer Research Meetings that will be held in San Francisco in April, 2002.

In addition, as noted above we have submitted several grants to do further investigations enhancing the already collected data from this breast cancer case-control study. Two grants have been funded; another, an R01 was resubmitted to the National Institutes of Health after receiving a score that was just outside the payline on a previous submission. The abstracts for each of those grants follow. The first, funded by the DoD, is an examination of residence at the time of birth and at menarche in relation to exposures from environmental contaminants and risk of breast cancer. This study utilizes a residential history that was part of the original interview completed by cases and controls. In addition to the work mapping both residences and industries described above, the study will include additional analyses of several genetic factors that are important in the metabolic handling of benzene and polycyclic aromatic hydrocarbons. The second study, funded by the National Cancer Institute, will examine residence in the period of life following menarche. Similar exposures and genetic factors will be examined. The third proposed study would include a focus on alcohol and the underlying mechanism for the association we see with risk. This grant would involve the collection of archived tumor blocks from the participants in our study. We propose to examine genetic factors, particular biomarkers in the tumor tissue as well as the interview data to examine two possible mechanisms to explain the observed associations of diet and breast cancer risk. Much of this study would include a particular focus on the mechanism for the observed alcohol association.

Environmental Exposures at Birth and at Menarche and Risk of Breast Cancer (funded by the DoD); Jo Freudenheim, P.I.

There is considerable evidence that environmental factors are important in the etiology of breast cancer. Breast cancer rates have changed markedly over time in genetically stable populations and there are important changes in rates for migrants from low risk to high-risk areas. Environmental factors related to industrialization may be important contributors to risk. Risk tends to be higher in more industrialized societies. There has been little study of proximity to industrial sites, to toxic waste sites and to other potentially toxic sources as potential risk factors. In particular, aromatic hydrocarbons such as benzene and some polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene may be important in relation to risk. Further, genetic variation may alter the association of exposure with risk. There is evidence that benzene exposure is affected by variation in the gene for NQO1. Metabolism of PAHs is affected by genetic variation in detoxification systems, including GST M1-1, GST P1-1 and cytochrome P450 1A1 (cyp1A1). Recently attention has focused on the infant period, early childhood and menarche as potentially sensitive periods of exposure. Breast tissue cell division is particularly rapid and therefore may be more sensitive to environmental insults. There are few studies examining these time periods; none have focused on

these exposures. We propose here to examine environmental exposures and gene-environment interactions at the time of birth and at menarche and subsequent risk of breast cancer.

We propose a population-based study to examine location of residence during these potentially sensitive time periods in relation to proximity to industrial sites, gasoline stations, toxic waste sites and heavily trafficked roadways as risk factors for subsequent disease. We expect that there will be an increase in risk for women who lived close to these sites during childhood, in particular, that point sources of benzene and PAHs will increase risk. Further, we wish to examine gene-environment interactions for these exposures experienced at birth and at menarche. The aims of the study are: 1) To investigate distance from steel mills, chemical factories, gasoline stations, toxic waste sites and other industrial sites of the residence of cases and controls at the time of birth and at menarche as risk factors for pre- and postmenopausal breast cancer, with control for appropriate confounders, and 2) To examine estimated exposure to benzene and to PAHs as risk factors for pre- and postmenopausal breast cancer, with control for the appropriate confounders. A secondary aim is: 3) To evaluate genetic susceptibility in relation to these exposures and breast cancer.

We will use data from an ongoing case-control study of breast cancer in Erie and Niagara Counties. We will collect residential histories, DNA for genotyping and data on other breast cancer risk factors for approximately 1000 cases of incident, primary, histologically-confirmed breast cancer and more than 2000 controls, age 35-79, frequency-matched to cases on age, race and county. Addresses for the women at the time of their birth and at menarche will be geocoded using a Geographic Information System (GIS). Historical data will be collected regarding location of steel mills, chemical factories, gasoline stations, toxic waste sites and other industrial sites during the period 1918-80. Distance between these sites and the residences of cases and controls at the time of birth and at menarche will be calculated using the GIS. More than 75% of participants in the original study lived in these counties at the time of their births. Molecular analysis will be performed at the Laboratory for Human Carcinogenesis of the NCI for the polymorphisms in NQO1, GSTM1-1, GST P1-1 and cyp 1A1. We will calculate odds ratios and 95% confidence intervals for distance from each category of potential exposure and for an index of probable level of exposure to PAHs and to benzene. We will also examine risk within categories stratified on genotype. The proposed study will contribute to our understanding of the role of environmental exposures during infancy and menarche. This is a unique and cost effective opportunity to examine a hypothesis of potentially great public health importance in a relatively residentially stable population.

<u>Breast Cancer Risk: Residential Environment and Genetics</u> (funded by NCI); Jo Freudenheim, P.I.

There is evidence that environmental factors related to industrialization may be important in breast cancer etiology. There has been little study of proximity to potentially toxic industrial sites as breast cancer risk factors. We propose to conduct a case-control study to examine location of residence during adult life in relation to breast cancer risk. The aims of the study are: 1) To investigate distance from steel mills, chemical factories, and other industrial sites of the residence as risk factors. The time periods to be examined will be (1)the primary residence during the period between menarche and first pregnancy (if any, otherwise menopause) and (2) residence(s) 10 and 20 years ago; 2) To examine estimated exposure to benzene and to PAHs based on residential exposure during these time periods as risk factors. Secondary objectives are: 3) To examine genetic variability in metabolism by NQO1, GST M1-1, GST P1-1 and CYP 1A1in relation to these exposures and breast cancer risk; 4) To evaluate all adult residences in relation to distance from potentially important exposures (steel mills, chemical factories, etc.) and risk; 5) To examine estimated exposure to benzene and to PAHs during the entire adult life and risk. We will use data from an ongoing case-control study of breast cancer in Erie and Niagara Counties including approximately 1000 cases of incident, primary, histologically-confirmed breast cancer and more than 2000 controls, age 35-79,

frequency-matched to cases. About 75% of participants in the original study lived in these counties at the time of their menarche. Addresses for the women at the time of their birth and at menarche will be geocoded using a Geographic Information System (GIS). Historical data will be collected regarding location of potentially important industrial sites. We will calculate odds ratios and 95% confidence intervals for distance from each category of potential exposure and for an index of probable level of exposure to PAHs and to benzene and we will examine risk within categories stratified on genotype. This is a unique and cost effective opportunity to examine a hypothesis of potentially great public health importance in a relatively residentially stable population.

Methylation and Oxidation in Breast Cancer Epidemiology (submitted to NCI), Jo Freudenheim, P.I.

There is considerable epidemiologic evidence that alcohol intake is related to risk of breast cancer and that intake of vegetables and fruits may reduce risk. Utilizing an existing case control study, we propose to examine two etiological mechanisms, one-carbon metabolism and/or oxidative stress and breast cancer. Our first aim is to examine the relation of elements related to one-carbon metabolism with risk. We propose a) to investigate genetic variation in enzymes important in one-carbon metabolism (methylene tetrahydrofolate reductase (MTHFR), methionine synthase (MS) and cystathione B-synthase (CBS)) in relation to risk and to investigate interaction of these genetic factors with dietary folate and alcohol with breast cancer risk; b) to investigate the association of dietary folate and alcohol and these genetic factors with total p53 mutations and with particular p53 mutations and c) to investigate the association of dietary folate and alcohol and these genetic factors with hypermethylation of the p16 gene, the BRCA1 gene and the estrogen receptor gene in breast tumors. Our second aim is to examine elements related to oxidative stress and antioxidants with risk. We propose to a) examine the relation of genetic variation in an enzyme important in the control of oxidative balance (manganese superoxide dismutase (MnSOD)) and to examine interactions of this genetic factor with dietary factors both oxidants and antioxidants; and b) to investigate the association between dietary sources of oxidants and antioxidants with total and particular p53 mutations. By combining information on intake, genetic susceptibility and tumor characteristics, it will be possible to make clearer inferences about the role of these two mechanisms in breast cancer etiology, with potentially important public health implications.

Summary

All field work has been completed for this case control study of breast cancer. The data set is almost ready for analysis. Additional studies using this data set are already in progress and another large one is planned. We are in the process of writing the manuscripts for the major hypotheses described in the original grant. In addition, we expect that this case control study will be a rich source for additional analyses. Some of these will focus on alcohol consumption because that was the focus of the original grant and of the original interview. We expect to look at exposures, genetic variation and different periods in the lifetime to get a better understanding of the relation of alcohol consumption to breast cancer risk. Further, the new proposed project will allow us to look at chemical characteristics of the tumors themselves with the possibility of making stronger connections of exposure and risk by examination of tumor characteristics. This work has important potential for public health in that it will provide understanding of the etiology of the alcohol and breast cancer association and perhaps insight into other factors which either increase or decrease the effect of alcohol on risk. In addition, this study has formed the basis for an environmental epidemiology effort to look at exposures throughout the lifetime as potentially explaining risk.

Because this study is relatively large in size it will be an important resource for studying both the originally planned analyses and for a large number of additional hypotheses.

(6) Key Research Accomplishments

- Interviewed a total of 1,181 women with primary, histologically-confirmed breast cancer
- Interviewed a total of 2,181 controls, frequency matched to cases on age, race and county of residence.
- Sent all remaining biological samples totaling 969 from breast cancer cases and 2,016 from controls to Dr. Shield's lab for DNA extraction. These samples represent all of the interviewed cases and controls for whom we had a biological sample. ADH3 analyses are ongoing.
- Data has been coded and entered for all interviews. Ongoing is the examination of data for mistakes, outliers and inconsistencies.
- Preliminary analyses of ADH3 and risk of breast cancer have been presented at the DoD Era of Hope meeting. These very preliminary findings appear to indicate that there is a group of premenopausal women who are genetically susceptible to an increased risk of breast cancer associated with alcohol consumption. These findings will be investigated in more depth when the interviewing is finished. Preliminary findings are included in this report for lifetime alcohol consumption and risk of breast cancer. We did not find increased risk of breast cancer in these preliminary analyses. There was some indication for increased risk for wine and beer consumption. We will examine these findings in more detail when the data set has been finalized.
- Two grants examining new hypotheses and utilizing this same data set have been funded. Another grant has been submitted that would focus on alcohol and diet and two potential underlying mechanisms of an observed association.

(7) Reportable Outcomes

1. Abstract presentation at DoD Era of Hope Meeting, ALCOHOL CONSUMPTION, ALCOHOL DEHYDROGENASE GENOTYPE AND BREAST CANCER RISK

2. Grants funded based on this research:
   - Environmental Exposures at Birth and at Menarche and Risk of Breast Cancer (funded by the DoD); Jo Freudenheim, P.I.
   - Breast Cancer Risk: Residential Environment and Genetics (funded by NCI); Jo Freudenheim, P.I.
3. Grants submitted based on this research:
   - Methylation and Oxidation in Breast Cancer Epidemiology (submitted to NCI), Jo Freudenheim, P.I.

(8) Conclusions

This study is a detailed examination of the association between alcohol and breast cancer, including an in-depth history of lifetime alcohol consumption including particular beverages, portions, drinking with or not with meals, pattern of drinking (i.e., differentiating occasional heavy drinking from consistent light drinking). We are also looking at how associations may differ by genetic susceptibility and by other risk factors including use of postmenopausal hormones, and by estrogen receptor status and histology for the cases. In that alcohol is one of the few easily modified risk factors that has been consistently identified for breast cancer, a clearer understanding of this risk factor is merited. In particular, if there are groups of women who are at particular risk for an effect of alcohol because of genetic susceptibility, this finding would have important public health implications. We will complete these analyses as soon as possible.

(9) References

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(10) Appendices

There are no appendices to include in this report.

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